# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)



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|---|---|---|
| Applicant's or agent's file reference 4647-044785 | FOR FURTHER ACTION | See Form PCT/IPEA/416 |
| International application No. PCT/US04/29007 | International filing date *(day/month/year)* 03 September 2004 (03.09.2004) | Priority date *(day/month/year)* 04 September 2003 (04.09.2003) |
| International Patent Classification (IPC) or national classification and IPC IPC(7): C12N 5/00, 5/02 and US Cl.: 435/375 | | |
| Applicant MEDTRAIN TECHNOLOGIES, LLC | | |

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 1 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☒ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

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|---|---|
| Date of submission of the demand 19 July 2005 (19.07.2005) | Date of completion of this report 08 November 2005 (08.11.2005) |
| Name and mailing address of the IPEA/ US<br>Mail Stop PCT, Attn: IPEA/US<br>Commissioner for Patents<br>P.O. Box 1450<br>Alexandria, Virginia 22313-1450<br>Facsimile No. (571) 273-3201 | Authorized officer<br>Patrick S. Riggins J. Roberts for<br>Telephone No. (571) 272-1600 |

Form PCT/IPEA/409 (cover sheet)(April 2005)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US04/29007

**Box No. I Basis of the report**

1. With regard to the **language**, this report is based on:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into English, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4(a))

☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:
pages 1-26 as originally filed/furnished
pages* NONE received by this Authority on
pages* NONE received by this Authority on

☒ the claims:
pages 28 and 29 as originally filed/furnished
pages* NONE as amended (together with any statement) under Article 19
pages* 27 received by this Authority on 19 July 2005 (19.07.2005)
pages* NONE received by this Authority on

☒ the drawings:
pages 1-16 as originally filed/furnished
pages* NONE received by this Authority on
pages* NONE received by this Authority on

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE
☒ the claims, Nos. 2 and 3
☒ the drawings, sheets/figs NONE
☒ the sequence listing (*specify*): NONE
☒ any table(s) related to the sequence listing (*specify*): NONE

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages
☐ the claims, Nos
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to the sequence listing (*specify*):

** If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
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**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | |
|---|---|---|
| Novelty (N) | Claims 4, 6, 7 and 11-18 | YES |
| | Claims 1, 5 and 8-10 | NO |
| Inventive Step (IS) | Claims 4-7, 11, 12, 17 and 18 | YES |
| | Claims 1, 5, 8-10 and 13-16 | NO |
| Industrial Applicability (IA) | Claims 1 and 4-18 | YES |
| | Claims NONE | NO |

2. Citations and Explanations (Rule 70.7)

Claims 1, 5, and 8-10 lack novelty under PCT Article 33(2) as being anticipated by Ruoslahti. The claims are drawn to a method for manipulating the intrinsic strain of cells by treating with at least one compound that will alter the intrinsic strain set point through a variety of means including affecting extracellular matrix organization or the ability of the cells to attach to that matrix, wherein the cells are an *in situ* native tissue or an *in vitro* fabricated tissue engineered construct. The compound can be a peptide that disrupts integrin binding to matrix components. The compound can be added at any point of the fabrication of the tissue engineered construct. Ruoslahti discloses the use of peptides that bind to integrins and block the ability of the integrins to bind to extracellular matrix proteins. At the bottom of column 6 through the top of column 7, Ruoslahti clearly contemplates treatment of *in situ* tissues for the prevention of metastasis or for enhancing wound healing in concert with a prosthetic device or being integrated into a matrix to be implanted. In the case of the wound healing contemplation, the compound is clearly added at the beginning of the fabrication, as the fabrication would seem to occur *in situ*.

Claims 1, 8-10, and 13-14 lack an inventive step under PCT Article 33(3) as being obvious over Ruoslahti in view of Holvoet. The claims are drawn to modulating intrinsic strain indirectly through up or down regulation of cytoskeletal genes or matrix metalloproteases through induction of TNF or IL-1. Ruoslahti teaches the limitations as described above but does not teach using TNF or IL-1 to alter cytoskeletal genes or matrix metalloproteases. Holvoet teaches that TNF upregulates MMP-9 which leads to extracellular matrix destruction. Therefore one would have known to modulate cell binding to extracellular matrix through destruction of the matrix by treating with TNF.

Claims 1, 8-10, and 15-16 lack an inventive step under PCT Article 33(3) as being obvious over Ruoslahti in view of Rotsch. Rotsch teaches that cytochalasin D leads to alterations in the modulus of the cell. Therefore as described above, one would have known to disrupt cell strain through treatment with cytochalasin D or through disruption of microtubules.

Claims 4, 6-7, 11-12, and 17-18 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest use of these methods when a mechanical strain is applied to the cells. Additionally, the prior art does not teach of using nucleotides, hyaluronic acid, antisense, or siRNA for modulating cell strain.

Claims 1 and 4-18 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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**Box No. VII Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

The description is objected to as containing the following defect(s) under PCT Rule 66.2(a)(iii) in the form or contents thereof: some references, e.g. on pages 20 and 21, are cited through an apparent end notation type of format, yet there is no listing of references to know what the reference numbers are referencing.

Claims 11 and 14 are objected to under PCT Rule 66.2(a)(iii) as containing the following defect(s) in the form or contents thereof: Claim 11 repeats uridine triphosphate. Claim 14 incorrectly identifies tumor necrosis factor as TGF.

**Box No. VIII Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 17 and 18 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because the claims are not fully supported by the description. The description does not disclose the claimed invention in a manner sufficiently clear and complete for the claimed invention to be carried out by a person skilled in the art because of the following reasons. The description goes into great detail attempting to establish the ease with which one can use antisense or siRNA. Though this technology would appear to be very well established, it is indeed necessary to attempt multiple embodiments for any gene of interest that one wishes to downregulate. As no genes have been specifically identified and no examples of successful use of antisense or siRNA have been presented one would be unable to practice the invention as delineated in claims 17 and 18 without a high level of trial and error type experimentation.

Claims 9, 12, 13, and 16 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): these claims all describe a required limitation and then exemplify with the phrase "such as". It is thus unclear if the exemplified embodiments that appear after "such as" are intended as limitations or not. Thus, one can not be certain what the metes and bounds of these claims compise.

THE INVENTION CLAIMED IS:

1. A method for manipulating the intrinsic strain of cells, comprising treating the cells either *in vivo* or *in vitro* with at least one compound that affects the intrinsic strain setpoint of the cells in order to modulate extracellular matrix synthesis, secretion, stiffness, organization and/or remodeling, or attachment of the cells to the matrix via integrins or other like cell-matrix attachments, wherein the cells comprise an in situ native tissue or an in vitro fabricated tissue engineered construct.

2-3 (Canceled)

4. The method according to claim 1, wherein the tissue engineered construct is a human tendon internal fibroblast (HTIF)-populated bioartificial tendon or other fibroblast from another connective tissue.

5. The method according to claim 1, wherein the compound is added at the beginning, during or at the end of fabrication of the tissue engineered construct.

6. The method according to claim 1, further comprising applying a mechanical external strain to the cells.

7. The method according to claim 6, wherein the mechanical external strain is comprised of uniaxially loading a tissue engineered construct by placing a loading post beneath a well of a culture plate and applying a vacuum to deform a flexible membrane downward so as to apply a uniaxial strain along a long axis of the tissue engineered construct.

8. The method according to claim 1, wherein the compound is a mediator which causes release of cell attachment points of the cells from its extracellular matrix.